09:42:45 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 11/

Project #: B-03-A07 Cost share #: Rev #: 2
Center # : 10/24-6-R6500-0A7 Center shr #: OCA file #: 12
Work type : RE
Contract#: F33615-87-D-0626-0007 Mod #: 03 Document : DO
Prime #: Contract entit

Subprojects ? : N
Main project #:

Project unit: BEC Unit code: 03.010.203
Project director(s):
TOLER J C BEC (404)894-3964

Sponsor/division names: AIR FORCE / WRIGHT-PATTERSON AFB,
Sponsor/division codes: 104 / 002

Award period: 890901 to 900701 (performance) 910131 (reports)

| Sponsor amount | New this change | Total to date |
|---|---|---|
| Contract value | 0.00 | 173,151.00 |
| Funded | 0.00 | 173,151.00 |
| Cost sharing amount | | 0.00 |

Does subcontracting plan apply ?: Y

Title: EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY

PROJECT ADMINISTRATION DATA

OCA contact: E. Faith Gleason 894-4820

| Sponsor technical contact | Sponsor issuing office |
|---|---|
| DR. JOHNATHAN L. KIEL<br>(512)536-3583 | JOHN M. LIPKER<br>(513)255-4818 |
| AIR FORCE SCHOOL OF<br>AEROSPACE MEDICINE<br>BROOKS AFB, TX 78235-5301 | ASD/PKRSC<br>AREA B, BUILDING 7<br>WRIGHT-PATTERSON AFB, OH 45433-6503 |

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): Y
Defense priority rating : DO-C9 GOVT supplemental sheet
Equipment title vests with: Sponsor X GIT

Administrative comments -
MOD. 03 LEAVES TECHNICAL PERFORMANCE PERIOD ENDING AT 7/1/90 BUT EXTENDS REPORTING PERIOD THROUGH 1/31/91.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

---

Closeout Notice Date 03/06/92

Project No. B-03-A07_____ Center No. 10/24-6-R6500-0A7_

Project Director TOLER J C_____ School/Lab BEC_____

Sponsor AIR FORCE/WRIGHT-PATTERSON AFB, OH_____

Contract/Grant No. F33615-87-D-0626-0007_____ Contract Entity GTRC

Prime Contract No. _____

Title EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY_____

Effective Completion Date 900701 (Performance) 910131 (Reports)

---

| Closeout Actions Required: | Y/N | Date Submitted |
|---|---|---|
| Final Invoice or Copy of Final Invoice | Y | _____ |
| Final Report of Inventions and/or Subcontracts | Y | _____ |
| Government Property Inventory & Related Certificate | Y | _____ |
| Classified Material Certificate | N | _____ |
| Release and Assignment | Y | _____ |
| Other _____ | N | _____ |

Comments_____
_____

Subproject Under Main Project No. _____

Continues Project No. _____

Distribution Required:

| | |
|---|---|
| Project Director | Y |
| Administrative Network Representative | Y |
| GTRI Accounting/Grants and Contracts | Y |
| Procurement/Supply Services | Y |
| Research Property Managment | Y |
| Research Security Services | N |
| Reports Coordinator (OCA) | Y |
| GTRC | Y |
| Project File | Y |
| Other _____ | N |
| _____ | N |

~~NOTE: Final Patent Questionnaire sent to PDPI~~.

B-10-A0

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: September 1, 1989 through December 31, 1989
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 0.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 0.00 |
| TOTAL | $ 0.00 | $ 0.00 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

Please forward approved "certificate" to:

Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-10-A0

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: January 1, 1990 through January 31, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 0.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 0.00 |
| TOTAL | $ 0.00 | $ 0.00 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

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Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-10-

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: February 01, 1990 through February 28, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 0.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 0.00 |
| TOTAL | $ 0.00 | $ 0.00 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

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Cruz Cantu

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Atlanta, Georgia 30332-0259

B-10-A0-

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: March 01, 1990 through March 31, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 0.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 0.00 |
| TOTAL | $ 0.00 | $ 0.00 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

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Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-10-A07

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: April 01, 1990 through April 30, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 0.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 0.00 |
| TOTAL | $ 0.00 | $ 0.00 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

_____
James C. Toler, Project Director

The above statement is approved for payment by the Government.

_____ _____
Johnathan L. Kiel Cruz Cantu

Please forward approved "certificate" to:

Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-10-A07

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: May 01, 1990 through May 31, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 94,968.00 | $ 94,968.00 |
| Management Fee (7.5% of Total Costs) | $ 7,122.60 | $ 7,122.60 |
| TOTAL | $ 102,090.60 | $ 102,090.60 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

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Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-03-A07

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-10-A00/R6500

Period Covered: June 01, 1990 through June 30, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 94,968.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 7,122.60 |
| TOTAL | $ 0.00 | $ 102,090.60 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

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Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B-03-A07

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-03-A00/R6500

Period Covered: July 01, 1990 through July 31, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 11,871.00 | $ 106,839.00 |
| Management Fee (7.5% of Total Costs) | $ 890.33 | $ 8,012.93 |
| TOTAL | $ 12,761.33 | $ 114,851.93 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

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Georgia Institute of Technology
Grants and Contracts Accounting
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Atlanta, Georgia 30332-0259

B-03-A0

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-03-A00/R6500

Period Covered: November 01, 1990 through November 30, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 142,452.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 10,683.90 |
| TOTAL | $ 0.00 | $ 153,135.90 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

_____
James C. Toler, Project Director

The above statement is approved for payment by the Government.

_____ _____
Johnathan L. Kiel Cruz Cantu

Please forward approved "certificate" to:

Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

B03-A07

CERTIFICATE OF SERVICES/RESEARCH
CONTRACT NO. F33615-87-D-0626
GEORGIA TECH NO. B-03-A00/R6500

Period Covered: December 01, 1990 through December 31, 1990
Delivery Order No. 0007

I. DIRECT COSTS:

| | Current | Cumulative |
|---|---|---|
| Subcontracts (University of Texas at San Antonio) | $ 0.00 | $ 142,452.00 |
| Management Fee (7.5% of Total Costs) | $ 0.00 | $ 10,683.90 |
| TOTAL | $ 0.00 | $ 153,135.90 |

"I certify that the above is a true and correct statement of efforts performed under Contract No. F33615-87-D-0626 by the Georgia Institute of Technology through the Georgia Tech Research Corporation for the subject time period."

James C. Toler, Project Director

The above statement is approved for payment by the Government.

Johnathan L. Kiel

Cruz Cantu

Please forward approved "certificate" to:

Georgia Institute of Technology
Grants and Contracts Accounting
Attn: Sandi Chestnut
Atlanta, Georgia 30332-0259

Georgia Tech

Georgia Institute of Technology
Centennial Research Building
400 Tenth Street, N.W.
Atlanta, Georgia 30332-0130

BIOENGINEERING CENTER
404•894•3964
FAX: 404•894•3120

January 8, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellott

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Academic Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject: Performance and Cost Report No. 1, Sequence No. 13, for the Reporting Period 1 September through 30 September 1989

Gentlemen:

Although the effective date of the Contract for Task 0007 is 1 September 1989, it was not officially received until 13 September 1989. The Request for Subcontract Agreement was completed on 18 September 1989 and delivered to the Georgia Tech Office of Contract Administration. On 22 September 1989, two copies of the subcontract agreement were submitted to the Office of Grants and Contracts at the University of Texas in San Antonio, TX. As of 30 September 1989, a fully executed subcontract agreement had not been received back from the University of Texas.

Since the subcontract for Task 0007 was still in negotiation, no technical performance was completed and no costs were incurred during this reporting period. Execution of the subcontract agreement is anticipated during the early part of the next reporting period, and Performance and Cost Reports will be forthcoming from Dr. Deborah Armstrong, the University of Texas Principal Investigator for this Task.

Respectfully submitted,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong
University of Texas

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
(404) 894-3964

**Norberto F. Ezquerra, Director**
Medical Informatics Laboratory
(404) 894-7026

**Philip R. Kennedy, Director**
Neuroscience Laboratory
(404) 894-4257

**Michael J. Sinclair, Director**
Robotics and Microelectronics Laboratory
(404) 894-4931

**KEY STAFF**

**David M. Banks**
(404) 894-7020

**Stephen J. Bonasera**
(404) 894-7031

**Michael F. Burrow**
(404) 894-7034

**John W. Peifer**
(404) 894-7028

**Wesley W. Shelton, Jr.**
(404) 894-8727

**Thomas G. Single**
(404) 894-7033

**Crystal L. Tucker**
(404) 894-7022

Georgia Tech

Georgia Institute of Tech
Centennial Research Buildi
400 Tenth Street, N.W.
Atlanta, Georgia 30332-020

BIOENGINEERING CENTE
404•894•3964
FAX: 404•894•3120

March 11, 1992

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Performance and Cost Reports, Sequence No. 13, for the October and November 1989 Reporting Periods

Gentlemen:

The requirement for the monthly Performance and Cost Report for October 1989 was overlooked by the subcontractor, Dr. Deborah Armstrong of the University of Texas/San Antonio. Efforts are underway to have this report prepared and submitted.

Technical and financial information that would have normally been included in the Performance and Cost Report for November 1989 was included instead in the first cumulative Performance and Cost Report submitted on January 8, 1990. The information in this first cumulative Performance and Cost Report covered performance on the subcontract for the months of September - November 1989.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

# Georgia Tech

Georgia Institute of Technology
Centennial Research Building
400 Tenth Street, N.W.
Atlanta, Georgia 30332-0130

BIOENGINEERING CENTER
404•894•3964
FAX: 404•894•3120

January 8, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellott

Reference: Task Order 0007 Under Contract No. F33615-87-D 0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Performance and Cost Report (Cumulative), Sequence No. 14, for the Reporting Period 1 September through 30 November 1989

Gentlemen:

Please find attached the contractually-required Performance and Cost Report (Cumulative) for the reporting period 1 September through 30 November 1989. This report was prepared and submitted by Dr. Deborah L. Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated with the College of Sciences and Engineering, Division of Life Sciences, at the University of Texas in San Antonio, TX.

Respectfully submitted,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong
University of Texas

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
(404) 894-3964

**Norberto F. Ezquerra, Director**
Medical Informatics Laboratory
(404) 894-7026

**Philip R. Kennedy, Director**
Neuroscience Laboratory
(404) 894-4257

**Michael J. Sinclair, Director**
Robotics and Microelectronics Laboratory
(404) 894-4931

**KEY STAFF**

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(404) 894-7020

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**John W. Peifer**
(404) 894-7028

**Wesley W. Shelton, Jr.**
(404) 894-8727

**Thomas G. Single**
(404) 894-7033

**Crystal L. Tucker**
(404) 894-7022

JAN 0 2 1990

THE UNIVERSITY OF TEXAS AT SAN ANTONIO
SAN ANTONIO, TEXAS 78285-0662
(512) 691-4458
FAX (512) 691-4510

COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

December 5, 1989

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Cost and performance Report for first 90 days after effective date of subcontract B-10-A07-S1 (September 1 - November 30, 1989)**
*University account number was issued on October 30, 1989

| | Current Period 9/1/89-11/30/89 | Cumulative 9/1/89-11/30/89 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 446 | 446 | 3,431 | 13.0% |
| Dollar Cost | | | | |
| Labor | $ 6,042.62 | $ 6,042.62 | $ 46,531 | 13.0% |
| Fringe | 664.74 | 664.74 | 12,098 | 5.5% |
| VSL | 0.00 | 0.00 | 110 | 0.0% |
| Indirect | 3,806.85 | 3,806.85 | 29,315 | 13.0% |
| Supplies | 2,292.83 | 2,292.83 | 13,276 | 17.3% |
| Equipment | 35,430.00 | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $48,237.04 | $48,237.04 | $158,280 | 30.5% |
| Percent of Work | 25% | 25% | | 25% |

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

Georgia Tech

Georgia Institute of Technolog
Centennial Research Building
400 Tenth Street, N.W.
Atlanta, Georgia 30332-0130

BIOENGINEERING CENTER
404•894•3964
FAX: 404•894•3120

March 29, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-062
"Pre-Acquired Research in Biotechnology", Georgia Tec
Project No. B-10-A07

Subject : Performance and Cost Report No. 4, Sequence No. 1
for the Period 1 December through 31 December 198

Gentlemen:

Please find attached the contractually-required December 198 Performance and Cost Report for Task 0007 under the reference contract. This report was prepared by Dr. Deborah Armstrong, th Principal Investigator on the Task 0007 subcontract Georgia Tech ha negotiated with the University of Texas at San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

CC: Dr. Armstrong

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
(404) 894-3964

**Norberto F. Ezquerra, Director**
Medical Informatics Laboratory
(404) 894-7026

**Philip R. Kennedy, Director**
Neuroscience Laboratory
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Robotics and Microelectronics Laboratory
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(404) 894-7022

THE UNIVERSITY OF TEXAS AT SAN ANTONIO
SAN ANTONIO, TEXAS 78285-0662
(512) 691-4458
FAX (512) 691-4510

OLLEGE OF SCIENCES AND ENGINEERING
vision of Life Sciences

January 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for Dec. 1, 1989 - Dec. 31, 1989 of subcontract B-10-A07-S1.**

| | Current Period 12/1/89-12/31/89 | Cumulative 9/1/89-12/31/89 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 240 | 680 | 3,431 | 20.0% |
| Dollar Cost | | | | |
| Labor | $ 6,789.05 | $12,831.67 | $ 46,531 | 27.0% |
| Fringe | 1,001.10 | 1,665.84 | 12,098 | 13.8% |
| VSL | 14.01 | 14.01 | 110 | 13.0% |
| Indirect | 4,344.99 | 8,151.84 | 29,315 | 27.0% |
| Supplies | 1,341.05 | 3,633.88 | 13,276 | 27.0% |
| Equipment | 35,430.00 | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $13,490.20 | $61,727.24 | $158,280 | 39.0% |
| Percent of Work | 8.3% | 33.2% | | 33.2% |

1/1/90

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

Georgia Tech

Georgia Institute of Technology
Centennial Research Building
400 Tenth Street, N.W.
Atlanta, Georgia 30332-0130

BIOENGINEERING CENTER
404•894•3964
FAX: 404•894•3120

March 29, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-062
"Pre-Acquired Research in Biotechnology", Georgia Tec
Project No. B-10-A07

Subject : Performance and Cost Report No. 5, Sequence No. 1
for the Period 1 January through 31 January 1990

Gentlemen:

Please find attached the contractually-required January 199 Performance and Cost Report for Task 0007 under the reference contract. This report was prepared by Dr. Deborah Armstrong, th Principal Investigator on the Task 0007 subcontract Georgia Tech ha negotiated with the University of Texas at San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. Armstrong

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
(404) 894-3964

**Norberto F. Ezquerra, Director**
Medical Informatics Laboratory
(404) 894-7026

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SAN ANTONIO, TEXAS 78285-0662
(512) 691-4458
FAX (512) 691-4510

OLLEGE OF SCIENCES AND ENGINEERING
ivision of Life Sciences

February 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for Jan. 1, 1990 - Jan. 31, 1990 of subcontract B-10-A07-S1.**

| | Current Period 1/1/90-1/31/90 | Cumulative 9/1/89-1/31/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 240 | 920 | 3,431 | 27.0% |
| Dollar Cost | | | | |
| Labor | $ 4,403.76 | $17,235.43 | $ 46,531 | 37.0% |
| Fringe | 915.18 | 2,581.02 | 12,098 | 21.0% |
| VSL | 7.01 | 21.02 | 110 | 19.0% |
| Indirect | 2,818.41 | 10,970.25 | 29,315 | 37.0% |
| Supplies | 1,581.73 | 5,215.61 | 13,276 | 39.0% |
| Equipment | -0- | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $ 9,726.09 | $68,634.92 | $158,280 | 43.0% |
| Percent of Work | 8.3% | 41.5% | | 41.5% |

2/1/90

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

Georgia Tech

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March 29, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-062
"Pre-Acquired Research in Biotechnology", Georgia Tec
Project No. B-10-A07

Subject : Performance and Cost Report No. 6, Sequence No. 1
for the Period 1 February through 31 February 1990

Gentlemen:

Please find attached the contractually-required February 199
Performance and Cost Report for Task 0007 under the reference
contract. This report was prepared by Dr. Deborah Armstrong, th
Principal Investigator on the Task 0007 subcontract Georgia Tech ha
negotiated with the University of Texas at San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

CC: Dr. Armstrong

**James C. Toler, Director**
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Co-Director, Bioengineering Center
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OLLEGE OF SCIENCES AND ENGINEERING
ivision of Life Sciences

March 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for Feb. 1, 1990 - Feb. 28, 1990 of subcontract B-10-A07-S1.**

| | Current Period 2/1/90-2/28/90 | Cumulative 9/1/89-2/28/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 240 | 1,160 | 3,431 | 34.0% |
| Dollar Cost | | | | |
| Labor | $ 4,820.37 | $22,055.80 | $ 46,531 | 47.0% |
| Fringe | 1,178.66 | 3,759.68 | 12,098 | 31.0% |
| VSL | -0- | 21.02 | 110 | 19.0% |
| Indirect | 3,085.03 | 14,055.28 | 29,315 | 47.0% |
| Supplies | 1,026.79 | 6,242.40 | 13,276 | 47.0% |
| Equipment | -0- | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $10,110.85 | $78,745.77 | $158,280 | 49.0% |
| Percent of Work | 8.3% | 50% | | 50.0% |

3/1/90

Deborah L. Armstrong
Associate Professor of Neurobiology
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March 29, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-062
"Pre-Acquired Research in Biotechnology", Georgia Tec
Project No. B-10-A07

Subject : Performance and Cost Report (Cumulative) No. 2
Sequence No. 14, for the Period 1 December 198
through 28 February 1990

Gentlemen:

Please find attached the contractually-required Performance and Cos
Report the time period 1 December 1989 through 28 February 1990 unde
Task 0007. This report was prepared by Dr. Deborah Armstrong, th
Principal Investigator under the Task 0007 subcontract negotiated b
Georgia Tech with the University of Texas at San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

CC: Dr. D. Armstrong

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Co-Director, Bioengineering Center
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OLLEGE OF SCIENCES AND ENGINEERING
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MAR 28 1990

March 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Quarterly Cost and Performance Report for Dec. 1, 1989 - Feb. 28, 1990 of subcontract B-10-A07-S1.**

| | Current Period 12/1/89-2/28/90 | Cumulative 9/1/89-2/28/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 714 | 1,160 | 3,431 | 34.0% |
| Dollar Cost | | | | |
| Labor | $16,013.18 | $22,055.80 | $ 46,531 | 47.0% |
| Fringe | 3,094.94 | 3,759.68 | 12,098 | 31.0% |
| VSL | 21.02 | 21.02 | 110 | 19.0% |
| Indirect | 10,248.43 | 14,055.28 | 29,315 | 47.0% |
| Supplies | 3,949.57 | 6,242.40 | 13,276 | 47.0% |
| Equipment | -0- | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $30,508.73 | $78,745.77 | $158,280 | 49.0% |
| Percent of Work | 25% | 50% | | 50.0% |

3/1/90

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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Atlanta, Georgia 30332-0130

Office of Interdisciplinary Programs
Bioengineering Center
(404) 894-3964

July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 00087 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Performance and Cost Report No. 7, Sequence No. 13, for the Reporting Period 1 March through 31 March 1990

Gentlemen:

Please find attached the March 1990 Performance and Cost Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong

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)LLEGE OF SCIENCES AND ENGINEERING
vision of Life Sciences

April 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for Mar. 1, 1990 - Mar. 31, 1990 of subcontract B-10-A07-S1.**

| | Current Period 3/1/90-3/31/90 | Cumulative 9/1/89-3/31/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 300 | 1,460 | 3,431 | 43.0% |
| Dollar Cost | | | | |
| Labor | $ 2,271.53 | $24,327.33 | $ 46,531 | 52.0% |
| Fringe | 590.58 | 4,350.26 | 12,098 | 36.0% |
| VSL | 7.01 | 28.03 | 110 | 25.5% |
| Indirect | 1,453.78 | 15,509.06 | 29,315 | 52.0% |
| Supplies | 2,086.64 | 8,329.04 | 13,276 | 62.7% |
| Equipment | -0- | 35,430.00 | 55,950 | 63.3% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $ 6,409.54 | $87,973.72 | $158,280 | 55.6% |
| Percent of Work period | 8.3% | 58.3% | | 58.3% |

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 00087 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Performance and Cost Report No. 8, Sequence No. 13, for the Reporting Period 1 April through 31 April 1990

Gentlemen:

Please find attached the April 1990 Performance and Cost Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong

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OLLEGE OF SCIENCES AND ENGINEERING
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May 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for Apr. 1, 1990 - 31, 1990 of subcontract B-10-A07-S1.**

| | Current Period 4/1/90-4/30/90 | Cumulative 9/1/89-4/30/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 300 | 1,720 | 3,431 | 50.0% |
| Dollar Cost | | | | |
| Labor | $ 2,271.53 | $26,598.86 | $ 46,531 | 57.0% |
| Fringe | 590.58 | 4,940.84 | 12,098 | 41.0% |
| VSL | -0- | 28.03 | 110 | 25.5% |
| Indirect | 1,453.78 | 16,962.84 | 29,315 | 57.0% |
| Supplies | 3,213.95 | 11,542.99 | 13,276 | 87.0% |
| Equipment | 20,500.00 | 55,930.00 | 55,950 | 100.0% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $28,029.84 | $116,003.56 | $158,280 | 73.3% |
| Percent of Contract | 8.3% | 66.6% | | 66.6% |

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 00087 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Performance and Cost Report No. 9, Sequence No. 13, for the Reporting Period 1 May through 31 May 1990

Gentlemen:

Please find attached the May 1990 Performance and Cost Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong

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)LLEGE OF SCIENCES AND ENGINEERING
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June 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Monthly Cost and performance Report for May 1, 1990 - May 31, 1990 of subcontract B-10-A07-S1.**

| | Current Period 5/1/90-5/31/90 | Cumulative 9/1/89-5/31/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 300 | 2,020 | 3,431 | 59.0% |
| Dollar Cost | | | | |
| Labor | $ 2,271.53 | $28,870.39 | $ 46,531 | 62.0% |
| Fringe | 590.58 | 5,531.42 | 12,098 | 46.0% |
| VSL | -0- | 28.03 | 110 | 25.5% |
| Indirect | 1,453.78 | 18,416.62 | 29,315 | 62.3% |
| Supplies | 1,114.00 | 12,656.99 | 13,276 | 95.3% |
| Equipment | -0- | 55,930.00 | 55,950 | 100.0% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $ 5,429.89 | $121,433.45 | $158,280 | 77.0% |
| Percent of Contract | 8.3% | 75% | | 75% |

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Cumulative Performance and Cost Report No. 3, Sequence No. 14, for the Reporting Period 1 March through 31 May 1990

Gentlemen:

Please find attached the March-through-May 1990 Cumulative Performance and Cost Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong

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OLLEGE OF SCIENCES AND ENGINEERING
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June 5, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Quarterly Cost and Performance Report for Mar. 1, 1989 - May 31, 1990 of subcontract B-10-A07-S1.**

| | Current Period 3/1/89-5/31/90 | Cumulative 9/1/89-5/31/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 900 | 2,060 | 3,431 | 59.0% |
| Dollar Cost | | | | |
| Labor | $ 6,814.59 | $28,870.39 | $ 46,531 | 62.0% |
| Fringe | 1,771.74 | 5,531.42 | 12,098 | 46.0% |
| VSL | 7.01 | 28.03 | 110 | 25.5% |
| Indirect | 4,361.34 | 18,416.62 | 29,315 | 62.3% |
| Supplies | 6,414.59 | 12,656.99 | 13,276 | 95.3% |
| Equipment | 20,500.00 | 55,930.00 | 55,950 | 100.0% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $30,508.73 | $121,433.45 | $158,280 | 77.0% |
| Percent of Work | 25% | 75% | | 75% |

Deborah L. Armstrong
Associate Professor of Neurobiology

Georgia Tech

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January 30, 1991

DEPARTMENT OF THE AIR FORCE
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. John Lipker

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-03-A07

Subject : Performance and Cost Report No. 10, Sequence No. 13, for the Reporting Period 1 June through 30 June 1990

Gentlemen:

Please find attached the June 1990 Performance and Cost Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong, Univ. of TX

**James C. Toler, Director**
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Co-Director, Bioengineering Center
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COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

Monthly Cost and performance Report for June 1, 1990 - June 30, 1990 of subcontract B-10-A07-S1.

| | Current Period 6/1/90-6/30/90 | Cumulative 9/1/89-6/30/90 | Total Budget | % Contract |
|---|---|---|---|---|
| Man Hours | 290 | 2,310 | 3,431 | 67.0% |
| Dollar Cost | | | | |
| Labor | $ 2,512.11 | $31,382.50 | $ 46,531 | 67.0% |
| Fringe | 688.90 | 6,220.32 | 12,098 | 51.0% |
| VSL | -0- | 28.03 | 110 | 25.5% |
| Indirect | 1,607.75 | 20,024.37 | 29,315 | 68.0 |
| Supplies | 60.00 | 12,716.99 | 13,276 | 96.0% |
| Equipment | -0- | 55,930.00 | 55,950 | 100.0% |
| Travel | 0.00 | 0.00 | 1,000 | 0.0% |
| Totals | $ 4,868.76 | $126,302.21 | $158,280 | 79.8% |
| Percent of Contract | 8.3% | 83.3% | | 83.3% |

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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January 2, 1990

DEPARTMENT OF THE AIR FORCE
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Research and Development Status Report (Cumulative), Sequence No. 1, for the Reporting Period 1 September through 30 November 1989

Gentlemen:

Please find attached the contractually-required Research and Development Status Report (Cumulative) for the reporting period 1 September through 30 November 1989. This report was prepared and submitted by Dr. Deborah L. Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated with the College of Sciences and Engineering, Division of Life Sciences, at the University of Texas in San Antonio, TX.

Respectfully submitted

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong
University of Texas

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
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Medical Informatics Laboratory
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)LLEGE OF SCIENCES AND ENGINEERING
vision of Life Sciences

December 5, 1989

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Research and Development Report for first 90 days after effective date of subcontract B-10-A07-S1 (September 1 - November 30, 1989)**

Procurement of Equipment and Supplies

An official account number was available by October 30, 1989 and purchase requests were immediately submitted for equipment and supplies essential for initiation of experiments. The following items have been ordered and anticipated arrival times range from the third to fourth weeks of December: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope. Some items are not identical to those listed on the subcontract budget due to the availability of improved models and competitive bidding.

Research Progress

Construction of antenna for irradiating neurons. Construction has been completed on a specialized antenna that will be mounted to a microscope stage for irradiating neurons in 35 mm culture dishes.

Immunohistochemical and morphological studies. Neurons in culture have been stained with specific antibodies to proteins specific for axonal, somatic and dendritic processes so that normal growth cone extensions can be characterized under control conditions. These data will be compared to growth characteristics of neurons exposed to RF radiation of various intensities and durations.

Electrophysiological studies. An experimental station for whole-cell and patch clamp recording of ionic conductance in single identified neurons in culture has been completed and electrode construction is being developed.

Optical recording experiments. Modifications of optical recording protocols are being made to enable integration of irradiation antenna and signal generation equipment into optical recording station.

We are eagerly anticipating the arrival of radiofrequency equipment so that irradiation experiments can begin.

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

Georgia Tech

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400 Tenth Street, N.W.
Atlanta, Georgia 30332-0130

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March 29, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffrey Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-062
"Pre-Acquired Research in Biotechnology", Georgia Tec
Project No. B-10-A07

Subject : Research and Development Status Report (Cumulative
No. 2, Sequence No. 1, for the Period 1 December 198
through 28 February 1990

Gentlemen:

Please find attached the contractually-required Performance and Cos
Report the time period 1 December 1989 through 28 February 1990 unde
Task 0007. This report was prepared by Dr. Deborah Armstrong, th
Principal Investigator under the Task 0007 subcontract negotiated b
Georgia Tech with the University of Texas at San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

CC: Dr. D. Armstrong

**James C. Toler, Director**
Bioelectromagnetics Laboratory
Co-Director, Bioengineering Center
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COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

MAR 26 1990

March 8, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Quarterly Research and Development Report for subcontract B-10-A07-S1 (December 1, 1989 - February 28, 1990)**

Completion of equipment set-up for initial irradiation experiments

The following instruments have been integrated into an experimental system for irradiating hippocampal neurons in culture: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope. The irradiation system can be interfaced with two types of antenna. The first is based on the design of Seiman, Burdette, and Dehaan (IEEE Transaction Microwave Theory and Techniques 37: 1989) and consists of a brass plate attached to a coaxial cable such that the center conductor is flush with the brass surface which provides support for the culture dishes. The second antenna is much smaller and can be inserted directly into the culture dish for irradiating single neurons during traditional or optical recording of neuronal activity during irradiation.

Research Progress

Several plates of freshly plated hippocampal neurons were placed on the larger brass antenna and were irradiated for four hours every 24 hours for three to four days. The carrier signal frequency was 400 MHz with an amplitude modulation of 16 Hz. Morphological examination revealed that cell viability was reduced in both irradiated and non-irradiated control plates and this effec

is believed to be related to poor temperature regulation of the plates while they are removed from the incubator and placed on the antenna. To correct for this problem we have now begun a series of experiments where the cells are irradiated within the incubator itself. Cells are constantly exposed to the RF field for four consecutive days and it appears that cell density gradients are developing, with a greater number of cells occurring in the center of the plate which is directly in the field as opposed to the more peripheral areas of the plate which are outside of the field. Non-irradiated control plates do not display this density gradient. This finding must be replicated and appropriate controls tested; however, we are very intrigued by this early indication of RF influence on fetal neuron development.

3/10/90

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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Office of Interdisciplinary Programs
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July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Research and Development Status Report (Cumulative) No. 3, Sequence No. 1, for the Reporting Period 1 February 1990 through 31 May 1990

Gentlemen:

Please find attached the February 1990 through May 1990 Research and Development Status Report (Cumulative) for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A07

cc: Dr. D. Armstrong

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COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

June 15, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**Quarterly Research and Development Report for subcontract B-10-A07-81 (February 28, 1990 - May 31, 1990)**

We have initiated a research program to examine the effects of radiofrequency electromagnetic radiation on cultured hippocampal neurons. The culture methodology was developed to facilitate utilizing whole-cell patch clamp and optical recording techniques in on-going experiments, and instruments needed for the generation, modulation and measurement of electromagnetic signals are in use in the laboratory.

The primary goal of these experiments is to systematically investigate the effects of radiofrequency radiation (RFR) on mammalian fetal neurons.

The following specific points are being examined:

1) Do fetal hippocampal neurons exposed to RFR display abnormal voltage and chemically-gated calcium currents?

2) Is the propagation of membrane potential changes or intracellular calcium fluctuations altered?

3) Are there changes in the concentration of proteins such as c-fos, tubulin, MAP-5, and, if so, is this do to changes in gene expression?

4) Is neurite growth-cone development and synaptic formation affected?

5) Are any observed effects temperature dependent, and if they are, what threshold intensities or critical exposure times exist?

6) If effects are due to athermal mechanism, are they dependent on specific carrier frequencies or extremely low frequency amplitude modulation of the carrier signal?

The following instruments have been integrated into an experimental system for irradiating hippocampal neurons in culture: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope. The irradiation system can be interfaced with two types of antenna. The first is based on the design of Burdette et al. (Dig. 1977 IEEE MTT-S Int. Microwave Symp, Pgs. 344-348) and consists of a brass plate attached to a coaxial cable such that the center conductor is flush with the brass surface which provides support for the culture dishes. The second antenna is much smaller and can be inserted directly into the culture dish for irradiating single neurons or neurites. A nonpurturbing Vitek temperature probe has been interfaced with an IBM PC to allow for mapping of temperature gradients in and around the electric field.

In preliminary experiments several dishes of freshly plated hippocampal neurons were placed on the larger brass antenna and were irradiated for four hours every 24 hours for three to four days. The carrier signal frequency was 147 MHz with an amplitude modulation of 16 Hz. Temperature increases within the field indicated an increase of less than 1 C and field strength was calculated to be 5 $mW/cm^2$. Morphological examination revealed that cell viability was reduced in both irradiated and non-irradiated control plates and this effect was attributed to poor temperature regulation of the plates while they are removed from the incubator and placed on the antenna. To correct for this problem we have now begun a series of experiments where the cells are irradiated within the incubator itself. Cells are constantly exposed to the RF field for four consecutive days and it appears that cell density gradients are developing, with a greater number of cells occurring in the center of the plate which is directly in the field as opposed to the more peripheral areas of the plate which are outside of the field. Non-irradiated control plates do not display this density gradient.

Summary of results for first six months of contract:

1. Density of neurons in center of irradiated plates (area within weak electric field) is greater than cell density at periphery of plate or in control plates. This density gradient is not dependent on ELF amplitude modulation.

2. Neurons in the field display normal neurite outgrowth and expression of proteins such as MAP-5.

3. Temperature within the electric field is approximately 1.5 to 2.5 degrees above the normal incubator temperature of 37 C.

4. Similar density gradients are not observed in temperature controls plates maintained at 39 C; however, some aggregation is apparent in plates maintained at 40 C. Neurons in temperature control plates maintained at 42 C display heat shock symptoms and normal neurite outgrowth is not observed.

Experiments are being conducted to determine if effects on neuron density gradients is the result of an aggregation phenomenon or increased cell division. Experiments determining calcium currents of under non-irradiated, temperature control conditions are being carried out.

Deborah L. Armstrong
Associate Professor of Neurobiology
Division of Life Sciences

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July 11, 1990

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. Jeffery Mellot

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-10-A07

Subject : Interim Technical Report, Sequence No. 18, for the Reporting Period 1 September 1989 through 28 February 1990

Gentlemen:

Please find attached the Interim Technical Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX.

Sincerely,

J.C. Toler, Director
Project No. B-10-A107

cc: Dr. D. Armstrong

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OLLEGE OF SCIENCES AND ENGINEERING
ivision of Life Sciences

June 29, 1990

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

**INTERIM TECHNICAL REPORT for Subcontract B-10-A07-S1, "Effects of Microwave Radiation on Neuronal Activity", (September 1, 1989 - February 28, 1990)**

We have initiated a research program to examine the effects of radiofrequency electromagnetic radiation on cultured hippocampal neurons. The culture methodology was developed to facilitate utilizing whole-cell patch clamp and optical recording techniques in on-going experiments, and instruments needed for the generation, modulation and measurement of electromagnetic signals are in use in the laboratory.

The primary goal of these experiments is to systematically investigate the effects of radiofrequency radiation (RFR) on mammalian fetal neurons.

The following specific points are being examined:

1) Do fetal hippocampal neurons exposed to RFR display abnormal voltage and chemically-gated calcium currents?

2) Is the propagation of membrane potential changes or intracellular calcium fluctuations altered?

3) Are there changes in the concentration of proteins such as c-fos, tubulin, MAP-5, and, if so, is this do to changes in gene expression?

4) Is neurite growth-cone development and synaptic formation affected?

5) Are any observed effects temperature dependent, and if they are, what threshold intensities or critical exposure times exist?

6) If effects are due to athermal mechanism, are they dependent on specific carrier frequencies or extremely low frequency amplitude modulation of the carrier signal?

The following instruments have been integrated into an experimental system for irradiating hippocampal neurons in culture: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope. The irradiation system can be interfaced with two types of antenna. The first is based on the design of Burdette et al. (Dig. 1977 IEEE MTT-S Int. Microwave Symp, Pgs. 344-348) and consists of a brass plate attached to a coaxial cable such that the center conductor is flush with the brass surface which provides support for the culture dishes. The second antenna is much smaller and can be inserted directly into the culture dish for irradiating single neurons or neurites. A nonpurturbing Vitek temperature probe has been interfaced with an IBM PC to allow for mapping of temperature gradients in and around the electric field.

In preliminary experiments several dishes of freshly plated hippocampal neurons were placed on the larger brass antenna and were irradiated for four hours every 24 hours for three to four days. The carrier signal frequency was 147 MHz with an amplitude modulation of 16 Hz. Temperature increases within the field indicated an increase of less than 1 C and field strength was calculated to be 5 $mW/cm^2$. Morphological examination revealed that cell viability was reduced in both irradiated and non-irradiated control plates and this effect was attributed to poor temperature regulation of the plates while they are removed from the incubator and placed on the antenna. To correct for this problem we have now begun a series of experiments where the cells are irradiated within the incubator itself. Cells are constantly exposed to the RF field for four consecutive days and it appears that cell density gradients are developing, with a greater number of cells occurring in the center of the plate which is directly in the field as opposed to the more peripheral areas of the plate which are outside of the field. Non-irradiated control plates do not display this density gradient.

Summary of results for first six months of contract:

1. Density of neurons in center of irradiated plates (area within weak electric field) is greater than cell density at periphery of plate or in control plates. This density gradient is not dependent on ELF amplitude modulation.

2. Neurons in the field display normal neurite outgrowth and expression of proteins such as MAP-5.

3. Temperature within the electric field is approximately 1.5 to 2.5 degrees above the normal incubator temperature of 37 C.

4. Similar density gradients are not observed in temperature controls plates maintained at 39 C; however, some aggregation is apparent in plates maintained at 40 C. Neurons in temperature control plates maintained at 42 C display heat shock symptoms and normal neurite outgrowth is not observed.

Experiments are being conducted to determine if effects on neuron density gradients is do to an aggregation phenomenon or increased cell division. Experiments determining calcium currents of under non-irradiated, temperature control conditions are being carried out.

## Experimental Design and Methods

### 1. Cell Culture Preparation.

Hippocampal neurons are prepared from 19-day-old fetuses in a procedure similar to that described by Banker and Cowan (J. Comp. Neur. 187: 469-494, 1979). The dam is anesthetized with Nembutal (60 mg/kg) and the fetuses removed and placed in chilled, sterile (4 C) buffered saline. Hippocampi are removed and incubated for 15 min at room temperature in Hank's balanced salt solution (Ca ,Mg -free) containing 0.1% trypsin. The hippocampi are then passed 10-15 times through a small bore, fire-polished Pasteur pipette and the resulting dissociated cells collected by centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) (GIBCO No. 430-1600 EB, NaHCO3-free) which contains 25 mM Hepes (ph 7.4), 10% fetal calf serum, 10% heat-inactivated horse serum, 100 ug/ml streptomycin, 100 units/ml penicillin G, 10ug/ml gentamicin sulfate, and 0.25 ug/ml amphotericin B. The cells are added to 35 mm Corning tissue culture dishes (at a density of one fetal hippocampus per two dishes) that have been treated for 2 hours with 0.1 mg/ml poly-L-lysine hydrobromide (30-70 kd, Sigma

Cell Culture Reagent) in water. After 2 hours at 37 C, the medium is replaced with one containing N-2 supplements (insulin, transferrin, putrecine, progesterone, sodium selenite). The cells are equilibrated with air rather than 5-10% CO2 and are placed in bicarbonate-free medium to repress the division of glia and allow easier observation of neurons. Using these procedures neurons have been maintained routinely for up to three weeks.

2. Irradiation procedures

The culture dish containing neurons is placed on the brass plate antenna which is attached to a 50 ohm coaxial cable such that the center conductor is flush with the brass plate and the bottom center of the dish. The center conductor diameter is 1.8 mm and the inner diameter of the outer conductor is 5.5 mm. The RF carrier frequency is provided by a HP 8644A signal generator and a low frequency signal from an HP function generator provides sinusoidal amplitude modulation. A linear microwave power amplifier (Airep Electronics) amplifies the signal. A dual directional coupler (HP 778D) attached to a dual sensor power meter (HP 438A) with sensors is used to measure the specific energy absorbed by the preparation using the method of Dutta et al. (Bioelectromagnetics 5: 71-78, 1884). The irradiation is conducted within an incubator. The initial RFR parameters are 147 Mhz carrier frequency amplitude modulated at 16 Hz since numerous studies have indicated alterations in calcium fluxes under these conditions, and our own preliminary studies revealed interesting gradients in cell density inside and outside the electric field generated under these conditions. Careful measurements of temperature gradients in and around the electric field will be conducted using the Vitek temperature probe to monitor thermal conditions so they can be minimized and appropriate controls designed. A second incubator is available for exposing half of the culture dishes from any given preparation to temperatures equivalent to those generated by the RFR. Initial exposures times will be long, 48 to 96 hours, beginning immediately after plating, when biological activity such as surface attachment, neurite outgrowth, and synaptic formation is maximized. Exposure times will be systematically varied to establish the existence of critical periods or thresholds. Irradiation will be carried out in the absence and presence of ELF modulation to determine if modulation is necessary in the alteration of cellular function. In some experiments a smaller antenna consisting of the flush cut end of smaller coaxial cable will be positioned in the culture dish to irradiate small groups of neurons on the microscope while growth cone

development and physiological parameters are monitored. Control data will be collected from two sources; neurons in the same dish that are outside of the center electric field and neurons from dishes that receive identical treatment except for the presence of RFR.

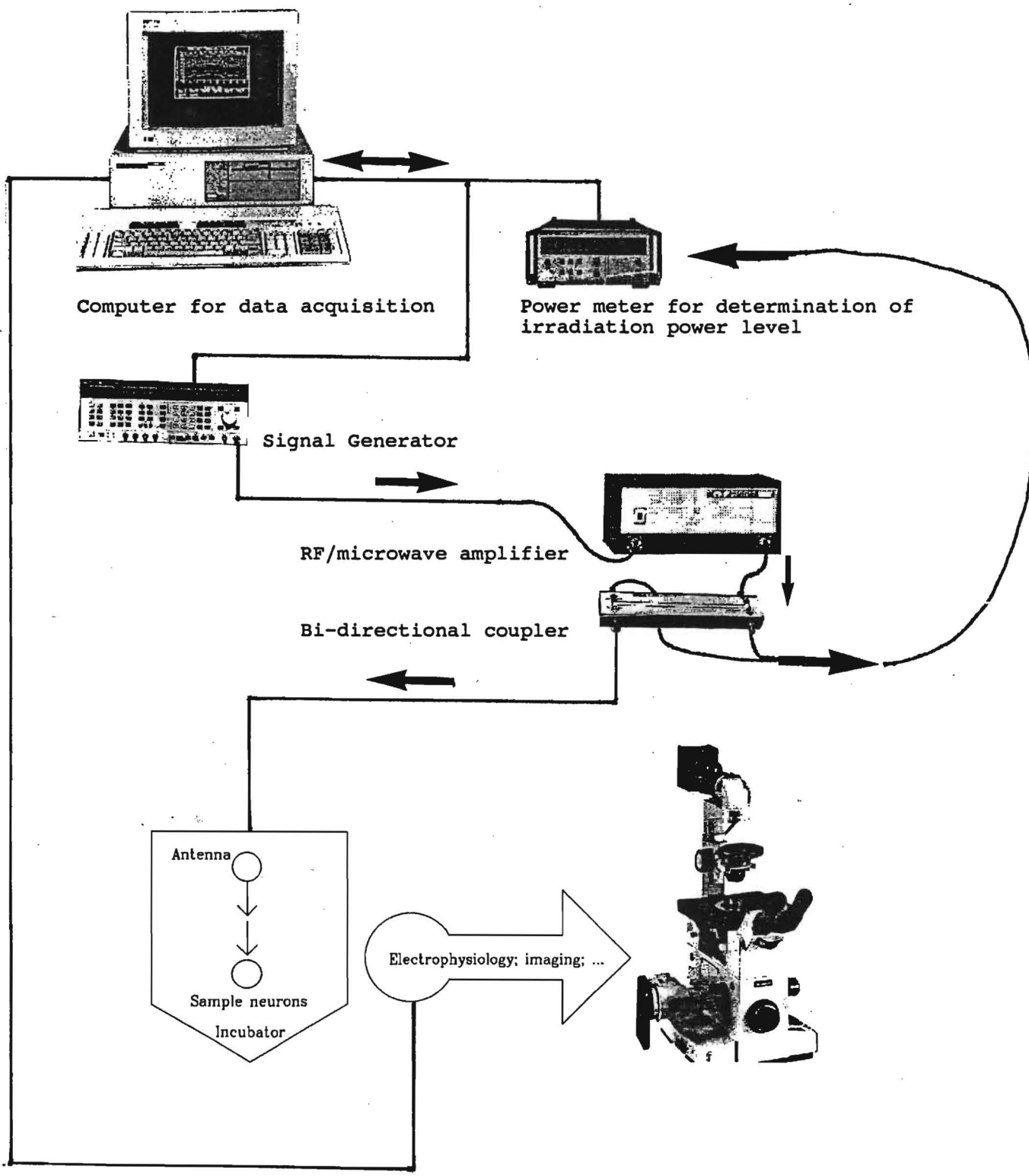
Deborah L. Armstrong
Associate Professor of Neurobiology

## APPENDIX

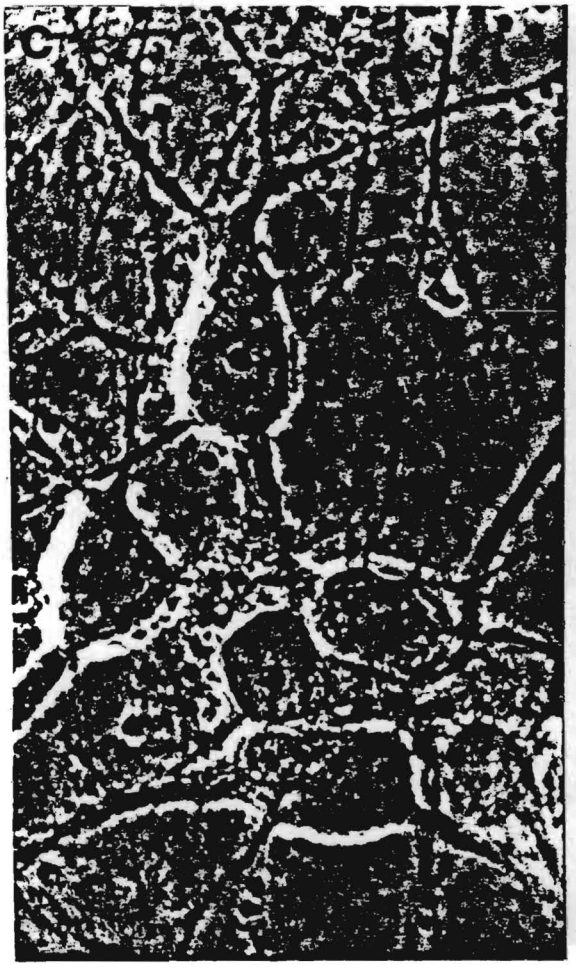
Figure 1. Diagram of irradiation equipment and its current arrangement during experiments. The microscope rests on an air table and is equipped with micromanipulators, and ports for CCD and 35 mm cameras or photodiode array.

Figure 2. Immunofluorescent detection of MAP5 in cultured hippocampal neurons. Phase contrast (a and c) and fluorescence (b and d) photomicrographs were made of neurons that had been in culture for two days (a and b) and 16 days (c and d). The cells were fixed using paraformaldehyde and were subsequently incubated with mouse monoclonal anti-MAP5 and fluorescein-labeled goat anti-mouse IgA. Bar = 14 um.

Fig. 1


Computer for data acquisition
Power meter for determination of irradiation power level
Signal Generator
RF/microwave amplifier
Bi-directional coupler
Antenna
Sample neurons
Incubator
Electrophysiology; imaging; ...


b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January 30, 1991

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**DEPARTMENT OF THE AIR FORCE**
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Attention: Mr. John M. Lipker

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-03-A07

Subject : Final Technical Report (Draft), Sequence No. 19, for the Reporting Period 1 September 1989 through 1 July 1990

Gentlemen:

Please find attached the draft version of the Final Technical Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah L. Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX. Dr. Armstrong's letter transmitting this report to Georgia Tech is attached for reference. Note that, in her letter, she states that prints for this report are still be developed.

Sincerely,

J.C. Toler, Director
Project No. B-03-A07

cc: Dr. Deborah Armstrong, Univ. of TX
Dr. Jonathan Kiel, USAFSAM/RZP

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COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

January 28, 1991

Mr. James C. Toler
OIP/BEC
Georgia Institute of Technology
Atlanta, Georgia 30332-0130

Dear Mr. Toler,

Enclosedpleasefindthefollowing Costand Performance Reports for Subcontract B-10-A07-S1: monthly for June 1, 1990 to January 31, 1991: and quarterly for June 1, 1990 to August 31, 1990, September 1, 1990 to November 30, 1990 and December 1, 1990 to January 31, 1991.

I have also enclosed a very, rough draft of the final report. Prints are still being developed, but I wanted to get something off to you. I would appreciate more information on the exact format for technical reports. A final report for evaluation will be following very shortly.

Sincerely,

Deborah L. Armstrong
Associate Professor
of Neurobiology

# EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY

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Draft Final Report for period Sep. 1, 1989- Jan. 31, 1991

# EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY

## INTRODUCTION

The medical uses of radiofrequency and microwave radiation, which encompasses electromagnetic waves in the frequency range of ten kilohertz (Khz) to 300 Gigahertz (GHz), include deep heating of muscles and cancer hyperthermia therapy (30). The possible biological effects of RFR at field strengths that produce minimal, or no, thermal changes has been addressed by recent articles in popular magazines, hence public interest has been piqued about the weak electric and magnetic fields that are ubiquitous in modern industrial environments. In particular, studies linking leukemia in children and residential proximity to power transformers (29), and reports of increased incidence of leukemia among electrical workers (22) have raised concerns. The purpose of this proposed research is not to resolve all of the questions that have been raised concerning the reliabilty of the health effects data, but rather to use current in vitro RFR exposure techniques on cultured fetal hippocampal neurons to detemine the response of these extremely active, developing cells to electromagnetic fields. It is recognized that mechanisms responsible for athermal biological responses have not been unequivocally defined, but data in the literature on neurite growth patterns (20,25-27) and increased protein synthesis (16,17), and ion fluxes (3,4,6,7) in electromagnetic fields provide compelling evidence that further research will elucidate important mecha-

nisms conderning how electromagnetic signals interact with cells.

It has been shown that exposure of diptera salivary glands and human cultured cells to extremely low-frequency (ELF) electromg-netic fields alters paterns of polypeptide synthesis (18,19). The effects displayed signal waveform dependence and only slight overlap with polypeptide synthesis produced by heat-shock conditions. Since increased neuronal expression of c-Fos immunoreactivity occurs after stress (9), a similar increase in immunoreactivity in neurons exposed to RFR would signify that the fields are producing a perturbation in the cells' environment that is being detected and transduced to the nucleus. Elevated intracellular calcium concentration is one mechanism of fos stimulation in neurons and should be investigated if RFR induces protein expression.

Several laboratories have focused on ionic fluctuations observed in the presense of ELF modulated RFR electromagnetic fields. Increased efflux of $^{45}Ca^{2+}$ from human neuroblastoma cells (IMR 32) occured during exposure to 147 MHz RFR that was amplitude modulated at ELF of 13, 16, 57.5 and 60 Hz (14). The effects were not linearly related to power density, in that significant changes occured only at specific absorption rates (SAR) of 0.005 and 0.05 (W/kg). These results agree with similar cell culture studies using 915 MHz RFR (13) and experiments using preparatins of fresh chick and cat brain tissue (3,4,6). The reported effects of RFR on calcium and other ions are varied and include both increased release, as discussed above, and increased uptake (15). Increased norepinephrine release has also been observed (12). In

reviewing these results one is promted to ask why only certain ELF modulations and "windows" of power density produce effects. Blackman et al. (8) discusses possible answers in terms of energy amplification and cooporativity processes that are set in motion so that membrane parameters, such as microvicosity, suddenly become extremely sensitive to small electromagnetic field disturbances. The entire issue of how very small energy levels of athermal RFR can significantly alter biological functions, when current intensities generated by cells are orders of magnitude larger, is one that has frequently been raised and has been addressed in depth by Adey (1). Several theoretical models have been proposed to explain the observed phenomena and the reader is referred to recent reviews (1,7,8).

Other effects that have been observed when cells are exposed to electric fields, in this case steady, uniform ones, include changes in alignment of fibroblasts (27) and accelerated neurite growth toward the cathode, or negative electrode (25). These responses are thought to be related to migration of membrane proteins, which has been demonstrated in muscle cells (21,24). It is not known if neurite alignment or synaptic densities are altered for neurons located inside, outside and at the edge of an electromagnetic field.

## METHODS AND PROCEDURES

### Experimental Animals

One pregnant female Sprague Dawley rat, purchased from Harlan

Sprague Dawley, was delivered every two weeks such that arrival date coincides with gestation day 15. The animal was housed in Isocages (Lab Products, Inc.) with white pine shaving litter at the temperature and humidity controlled University Animal Life Facility. A 12 hr light/dark schedule was maintained and food (Wayne Lab Blocks) and water were available ad libitum.

## Cell Culture Preparation.

Hippocampal neurons were prepared from 19-day-old fetuses in a procedure similar to that described by Bakker and Cowan (2). The dam was anesthesized with Nembutal (60 mg/kg) and the fetuses removed and placed in chilled, sterile (4 C) buffered saline. Hippocampi were removed and incubated for 15 min at room temperature in Hank's balanced salt solution ($Ca^{++}$,$Mg^{++}$ -free) containing 0.1% trypsin. The hippocampi were then passed 10-15 times through a small bore, fire-polished Pasteur pipette and the resulting dissociated cells collected by centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) (GIBCO No. 430-1600 EB, NaHCO3-free) which contained 25 mM Hepes (ph 7.4), 10% fetal calf serum, 10% heat-inactivated horse serum, 100 ug/ml streptomycin, 100 units/ml penicillin G, 10ug/ml gentamicin sulfate, and 0.25 ug/ml amphotericin B. The cells were added to 35 mm Corning tissue culture dishes (at a density of one fetal hippocampus per two dishes) that were treated for 2 hours with 0.1 mg/ml poly-L-lysine hydrobromide (30-70 kd, Sigma Cell Culture Reagent) in water. After 2 hours at 37C, the medium was be

replaced with one containing N-2 supplements (insuline, transferrin, putrecine, progesterone, sodium selenite). The cells were equilibrated with air rather than 5-10% CO2 and were placed in bicarbonate-free medium to repress the division of glia and allow easier observation of neurons. Using these procedures neurons have been maintained routinely for up to three weeks.

## Irradiation procedures

The culture dish containing neurons were placed on the brass plate antenna which is attached to a 50 ohm coaxial cable such that the center conductor is flush with the brass plate and the bottom center of the dish. The center conductor diameter is 1.8 mm and the inner diameter of the outer conductor is 5.5 mm. The RF carrier frequency was provided by a HP 8644A signal generator and a low frequency signal from an HP function generator provided sinusoidal amplitude modulation. A linear microwave power amplifier (Airep Electronics) amplified the signal. A dual directional coupler (HP 778D) attached to a dual sensor power meter (HP 438A) with sensors were used to measure the specific energy absorbed by the preparation using the method of Dutta et al. (13). The irradiation was conducted within the incubator. In some experiments a smaller antenna consiting of the flush cut end of smaller coaxial cable was positioned in the culture dish. Control data was collected from two sources; neurons in the the same dish that are outside of the center electric field and neurons from dishes that receive identical treatment except for the presence of RFR.

## RESULTS

The following instruments were been integrated into an experimental system for irradiating hippocampal neurons in culture: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope (Fig. 1). The irradiation system is interfaced with two types of antenna. The first is based on the design of Seaman, Burdette, and Dehaan (28) and consists of a brass plate attached to a coaxial cable such that the center conductor is flush with the brass surface which provides support for the culture dishes. The second antenna is much smaller and can be inserted directly into the culture dish for irradiating single neurons or neurites. A nonperturbing Vitek temperature probe was interfaced with an IBM PC to allow for mapping of temperature gradients in and around the electric field.

In preliminary experiments several dishes of freshly plated hippocampal neurons were placed on the larger brass antenna and were irradiated for four hours every 24 hours for three to four days. The carrier signal frequency was 400 MHz with an amplitude modulation of 16 Hz. Morphological examination revealed that cell viability was reduced in both irradiated and non-irradiated control plates. This effect was attributed to poor temperature regulation of the plates while they are removed from the incubator and placed on the antenna. Currently all cells are irradiated within the incubator itself. Culture dishes continuously exposed

to the RF field for four consecutive days develop density gradients, with a greater number of cells occurring in the center of the plate which is directly in the field as opposed to the more peripheral areas of the plate which are outside of the field. Non-irradiated control plates do not display this density gradient (Fig. 2). This finding has been replicated under various exposure periods and we have found that the density gradient is established within 20 min of placing the plates on the antennae.

## REFERENCES

1. Adey, W.R. (1981) Tissue interactions with nonionizing electromagnetic fields. Physiological Reviews 61:2:435-514.

2. Banker, G.A. and W.M. Cowan. (1979) Further observations on hippocampal neurons in dispersed cell culture. J. Comp Neur. 187:469-494.

3. Bawin, S.M. and W.R. Adey. (1976) Sensitivity of calcium binding in cerebral tissue to weak environment electric fields oscillating at low frequency. Proc. Natl. Acad. Sci. USA 73:6:1999-2003.

4. Blackman, C.F., S.G. Benane, D.E. House, and W.T. Joines. (1985) Effects of ELF(1-120Hz) and modulated (50Hz) RF Fields on the efflux of calcium ions from brain tissue in vitro. Bioelectromagnetics 6:1-11.

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June 13, 1991

**DEPARTMENT OF THE AIR FORCE**
Air Force Systems Command
Aeronautical Systems Division/PMRSC
Wright-Patterson AFB, OH 45433-6503

Attention: Mr. John M. Lipker

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-03-A07

Subject : Final Technical Report (Draft No. 2), Sequence No. 19, for the Reporting Period 1 September 1989 through 1 July 1990

Gentlemen:

Please find attached the 2nd draft of the Final Technical Report for the referenced Task Order. This report was prepared and submitted by Dr. Deborah L. Armstrong, the Principal Investigator on the subcontract Georgia Tech has negotiated for this Task with the University of Texas in San Antonio, TX. Dr. Armstrong's letter transmitting this report to Georgia Tech is attached for reference. Note that, in her letter, she states that prints for this report are still be developed.

Sincerely,

J.C. Toler, Director
Project No. B-03-A07

cc: Dr. Deborah Armstrong, Univ. of TX
Dr. Johnathan Kiel, USAFSAM/RZP

# EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY

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Draft Final Report for period Sep. 1, 1989- Jan. 31, 1991

ABSTRACT

The effects of radiofrequency radiation on rat hippocampal fetal neurons were examined. Carrier frequencies of 300 to 470 MHz were used, with most experiments carried out at 400 MHz. In some experiments the amplitude of the RF carrier was modulated, and the amplitude modulated frequency ranged from 10 Hz to 300 Hz. Neurons exposed to the RF field for any period up to 14 days appeared to be perfectly normal morphologically using phase contrast microscopy. Temperature measurements using a vitek probe revealed that culture dish temperature was maximum directly over the axis of symmetry of the antenna, but never exceeded 3° centigrade above the ambient temperature. A reliable effect observed in irradiated dishes was a concentration of neurons within the RF field. This density gradient was established within 20 min of placing the dish on the antennae, but did not occur if cells were allowed to attach to the dish prior to irradiation. The concentration phenomenon was not specific to neurons and was observed in both HeLa and CHO cell cultures. The evidence does not support changes in division rate or increased survival rate as explanations of the density gradient. A likely mechanism is that thermal convection currents within the irradiated dishes increases the probability of cells settling and attaching to the dish surface directly above the antenna. This technique is currently being exploited as a means of concentrating neurons within a small area to facilitate investigation of neural networks.

# EFFECTS OF MICROWAVE RADIATION ON NEURONAL ACTIVITY

## INTRODUCTION

The medical uses of radiofrequency and microwave radiation, which encompasses electromagnetic waves in the frequency range of ten kilohertz (Khz) to 300 Gigahertz (GHz), include deep heating of muscles and cancer hyperthermia therapy (30). The possible biological effects of RFR at field strengths that produce minimal, or no, thermal changes has been addressed by recent articles in popular magazines, hence public interest has been piqued about the weak electric and magnetic fields that are ubiquitous in modern industrial environments. In particular, studies linking leukemia in children and residential proximity to power transformers (29), and reports of increased incidence of leukemia among electrical workers (22) have raised concerns. The purpose of this proposed research is not to resolve all of the questions that have been raised concerning the reliability of the health effects data, but rather to use current in vitro RFR exposure techniques on cultured fetal hippocampal neurons to determine the response of these extremely active, developing cells to electromagnetic fields. It is recognized that mechanisms responsible for athermal biological responses have not been unequivocally defined, but data in the literature on neurite growth patterns (20,25-27) and increased protein synthesis (16,17), and ion fluxes (3,4,6,7) in electromagnetic fields

provide compelling evidence that further research will elucidate important mechanisms concerning how electromagnetic signals interact with cells.

It has been shown that exposure of diptera salivary glands and human cultured cells to extremely low-frequency (ELF) electromagnetic fields alters patterns of polypeptide synthesis (18,19). The effects displayed signal waveform dependence and only slight overlap with polypeptide synthesis produced by heat-shock conditions. Since increased neuronal expression of c-Fos immunoreactivity occurs after stress (9), a similar increase in immunoreactivity in neurons exposed to RFR would signify that the fields are producing a perturbation in the cells' environment that is being detected and transduced to the nucleus. Elevated intracellular calcium concentration is one mechanism of fos stimulation in neurons and should be investigated if RFR induces protein expression.

Several laboratories have focused on ionic fluctuations observed in the presence of ELF modulated RFR electromagnetic fields. Increased efflux of $Ca^{45}$ $^{2+}$ from human neuroblastoma cells (IMR 32) occurred during exposure to 147 MHz RFR that was amplitude modulated at ELF of 13, 16, 57.5 and 60 Hz (14). The effects were not linearly related to power density, in that significant changes occurred only at specific absorption rates (SAR) of 0.005 and 0.05 (W/kg). These results agree with similar cell culture studies using 915 MHz RFR (13) and experiments using preparations of fresh chick and cat brain tissue (3,4,6).

The reported effects of RFR on calcium and other ions are varied and include both increased release, as discussed above, and increased uptake (15). Increased norepinephrine release has also been observed (12). In reviewing these results one is prompted to ask why only certain ELF modulations and "windows" of power density produce effects. Blackman et al. (8) discusses possible answers in terms of energy amplification and cooporativity processes that are set in motion so that membrane parameters, such as microviscosity, suddenly become extremely sensitive to small electromagnetic field disturbances. The entire issue of how very small energy levels of athermal RFR can significantly alter biological functions, when current intensities generated by cells are orders of magnitude larger, is one that has frequently been raised and has been addressed in depth by Adey (1). Several theoretical models have been proposed to explain the observed phenomena and the reader is referred to recent reviews (1,7,8).

Other effects that have been observed when cells are exposed to electric fields, in this case steady, uniform ones, include changes in alignment of fibroblasts (27) and accelerated neurite growth toward the cathode, or negative electrode (25). These responses are thought to be related to migration of membrane proteins, which has been demonstrated in muscle cells (21,24). It is not known if neurite alignment or synaptic densities are altered for neurons located inside, outside and at the edge of an electromagnetic field.

## METHODS AND PROCEDURES

### Experimental Animals

One pregnant female Sprague Dawley rat, purchased from Harlan Sprague Dawley, was delivered every two weeks such that arrival date coincides with gestation day 15. The animal was housed in Isocages (Lab Products, Inc.) with white pine shaving litter at the temperature and humidity controlled University Animal Life Facility. A 12 hr light/dark schedule was maintained and food (Wayne Lab Blocks) and water were available ad libitum.

### Cell Culture Preparation.

Hippocampal neurons were prepared from 19-day-old fetuses in a procedure similar to that described by Bakker and Cowan (2). The dam was anesthetized with Nembutal (60 mg/kg) and the fetuses removed and placed in chilled, sterile (4 C) buffered saline. Hippocampi were removed and incubated for 15 min at room temperature in Hank's balanced salt solution ($Ca^{++}$ ,$Mg^{++}$ -free) containing 0.1% trypsin. The hippocampi were then passed 10-15 times through a small bore, fire-polished Pasteur pipette and the resulting dissociated cells collected by centrifugation and resuspended in Dulbecco's modified Eagle's medium (DMEM) (GIBCO No. 430-1600 EB, NaHCO3-free) which contained 25 mM Hepes (ph 7.4), 10% fetal calf serum, 10% heat-inactivated horse serum, 100 ug/ml streptomycin, 100 units/ml penicillin G, 10ug/ml gentamicin sulfate, and 0.25 ug/ml amphotericin B. The cells

were added to 35 mm Corning tissue culture dishes (at a density of one fetal hippocampus per two dishes) that were treated for 2 hours with 0.1 mg/ml poly-L-lysine hydrobromide (30-70 kd, Sigma Cell Culture Reagent) in water. After 2 hours at 37°C, the medium was be replaced with one containing N-2 supplements (insulin, transferrin, putrecine, progesterone, sodium selenite). The cells were equilibrated with air rather than 5-10% CO2 and were placed in bicarbonate-free medium to repress the division of glia and allow easier observation of neurons. Using these procedures neurons have been cultured for up to 30 days with a change of one-half of the medium every seven days.

## Irradiation procedures

The culture dish containing neurons were placed on the brass plate antenna which is attached to a 50 ohm coaxial cable such that the center conductor is flush with the brass plate and the bottom center of the dish. The center conductor diameter is 1.8 mm and the inner diameter of the outer conductor is 5.5 mm. The RF carrier frequency was provided by a HP 8644A signal generator. The carrier was amplitude modulated by mixing with either an HP 8644A internal wave or with a low frequency signal from an HP function generator. A linear microwave power amplifier (Airep Electronics) amplified the signal. Transmitted and reflected powers were measured via a dual directional coupler (HP 778D) connected to a dual sensor power meter (HP 438A), which coupled directly to the output of the Airep amplifier (13). The irradia-

tion was conducted within the incubator. Control data was collected from two sources; neurons in the same dish that are outside of the center electric field and neurons from dishes that receive identical treatment except for the presence of RFR.

A vitek four-conductor probe was used to measure the temperature of the irradiated medium. The vitek probe has two leads for powering the device and two leads (output leads) for observing the voltage across the thermistor that is incorporated into the tip of the device. In different experiments the vitek probe was powered by either a 3 volt battery or 5.0 volt/1000 Hz sinusoidal voltage applied across its two power leads. The voltage across the temperature sensitive resistor in the probe was measured by monitoring the potential across the two output leads. These high resistance leads were connected to an ultra-high input impedance quad JFET integrated circuit (IC) amplifier (LF347), which was used to buffer the signal. The two buffered signals were then fed into the inverting/non-inverting inputs of a high precision instrumentation amplifier IC (AD624) in order to take the difference of the two inputs and amplify the resultant. In the case of the sinusoidally driven probe, the signal was synchronously demodulated using an Analog Devices 630 IC and the output was low-pass filtered to extract the 1000 Hz component of the original unmodulated signal. The advantage of this approach is that thermal DC drifts in the electronics, 1/f noise and the line noise pick-up are filtered out. The processed voltage was fed into an AD389 sample and hold amplifier, which was then digitized using an ADC72 16-bit analog-to-digital

converter. An IBM PC interface was constructed (with DMA capability). Software was written (versions in assembler, FORTH and C) to program the IBM PC interface and collect the temperature-dependent voltage data. Data reduction programs were written in C to extract temperature from voltage data. 16-bit temperature data were collected, analyzed and stored on a hard disk.

A precision temperature reference was nor available for calibration of the probe to a $\pm 0.02^{\circ}$ C standard. Using less accurate equipment, the probe was calibrated to a $\pm 0.3^{\circ}$ C standard. Future accuracy will easily be increased by at least an order of magnitude with the addition of a precision temperature reference to the laboratory.

The carrier frequencies used were in the high RF range and varied from 300 Mhz to 470 MHz, with most experiments carried out at 400 MHz. In some experiments the amplitude of the high RF carrier was modulated, and the amplitude modulated frequency ranged from a frequency of 10 Hz to 300 Hz. Most experiments were carried out with no modulation of amplitude. During irradiation, transmitted and reflected powers were measured with a HP 438A power meter. This instrument has a GPIB (=HPIB) computer interface. The power meter was connected to an IBM RT, also equipped with a GPIB interface. A C program was written to periodically poll the power meter and obtain transmitted and reflected power measurements as a function of time. The computer signaled an alarm in the event of a reduction or failure of transmitted power.

# RESULTS

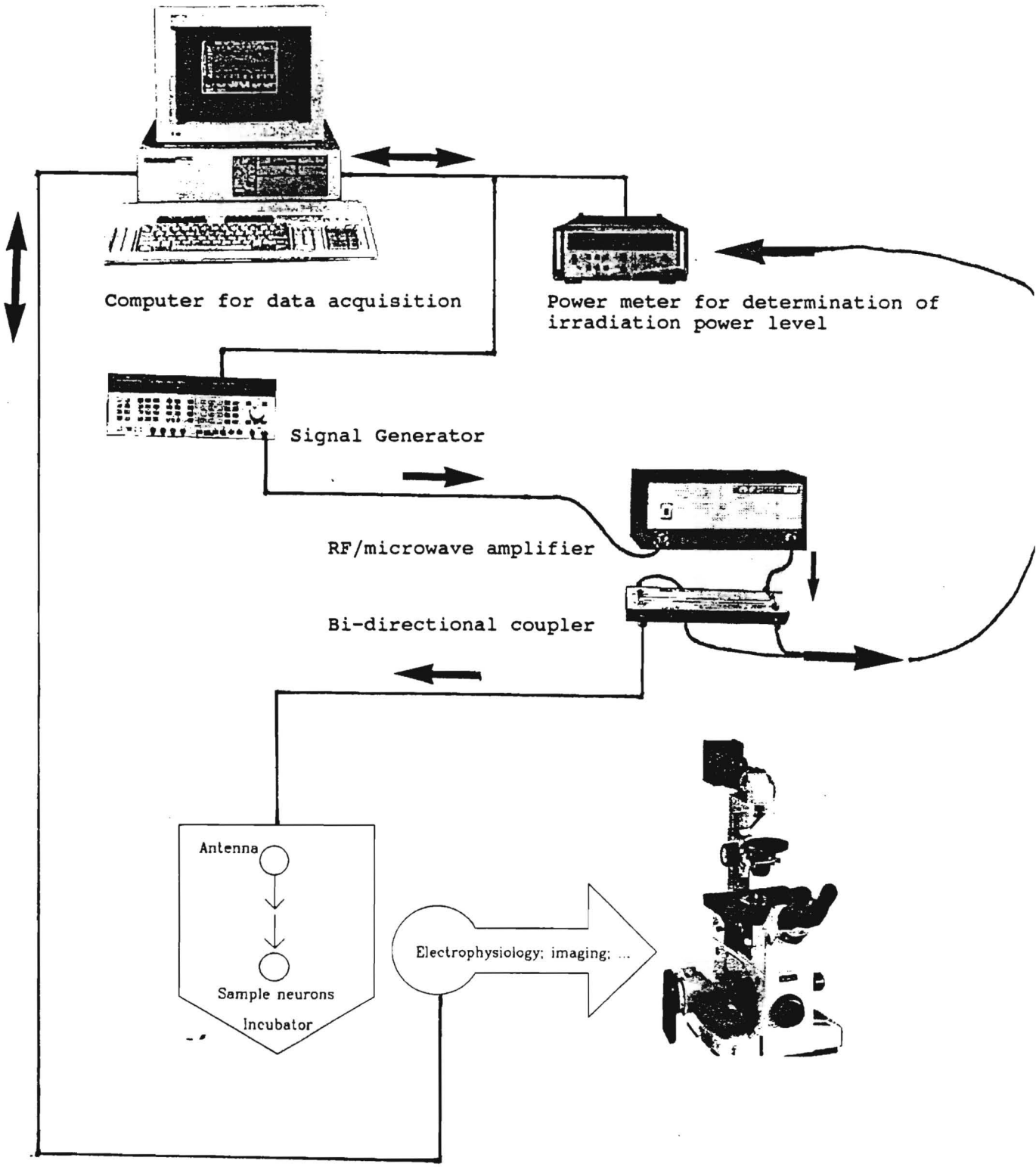
## Irradiation System

The following instruments were integrated into an experimental system for irradiating hippocampal neurons in culture: HP 8644A signal generator, HP 438A dual sensor power meter with sensors, HP 778D microwave dual directional coupler, Airep Electronics microwave power amplifier, HP 54502A digitizing oscilloscope (Fig. 1). The antenna is based on the design of Seaman, Burdette, and Dehaan (28) and consists of a brass plate attached to a coaxial cable such that the center conductor is flush with the brass surface which provides support for the culture dishes. A nonperturbing Vitek temperature probe was interfaced with an IBM PC to allow for mapping of temperature gradients in and around the electric field.

## Temperature Changes During Irradiation

As might be expected, it was found that the temperature of an irradiated sample was maximum at the center of the dish, directly on the axis of symmetry of the antenna, and fell off steeply to the background temperature as one moved off of the

Figure 1. Diagram of irradiation equipment and integration with cell culture incubator. The microscope rests on an air table and is equipped with micromanipulators, 35 mm camera, and video camera port.


Computer for data acquisition
Power meter for determination of irradiation power level
Signal Generator
RF/microwave amplifier
Bi-directional coupler
Antenna
Sample neurons
Incubator
Electrophysiology; imaging; ...

symmetry axis by one mm. The maximum never exceeded 3° centigrade degrees above the ambient temperature. This temperature change was independent of the carrier frequency and/or modulation used in these experiments.

## Characteristics of Cultured Neurons

The rat fetal hippocampus contains a total of 900,000 pyramidal neurons (2). Since we placed the hippocampal tissue from one fetus on two plates, we applied 450,000 pyramidal cells to each 35 mm culture dish. Our results agree with those of Banker et al. (2) in that 90% of the applied pyramidal cells attach to the dish. These neurons are derived mainly from areas CA1 and CA3 of the fetal hippocampus. Dentate gyrus granule cells are smaller (soma diameter = 10 um) and do not attach to the polylysine-coated substratum. They remain in suspension and do not extend neurites in our culture system. The detectable glia are flat, polygonal Type I and star-shaped, Type II astrocytes. These cells stain positively with antibody to the astrocyte marker protein, glial fibrillary acidic protein (GFAP). Other glial cells (oligodendrocytes, ependymal cells, microglia) do not survive under our culture conditions. The pyramidal cells are positively stained with antibody to the neuronal marker MAP5 (microtubule-associated protein 5), which is also called MAP1B. The pyramidal cells begin to extend neurites as soon as the cells attach to the substratum, which occurs within 30 minutes after application to the dishes.

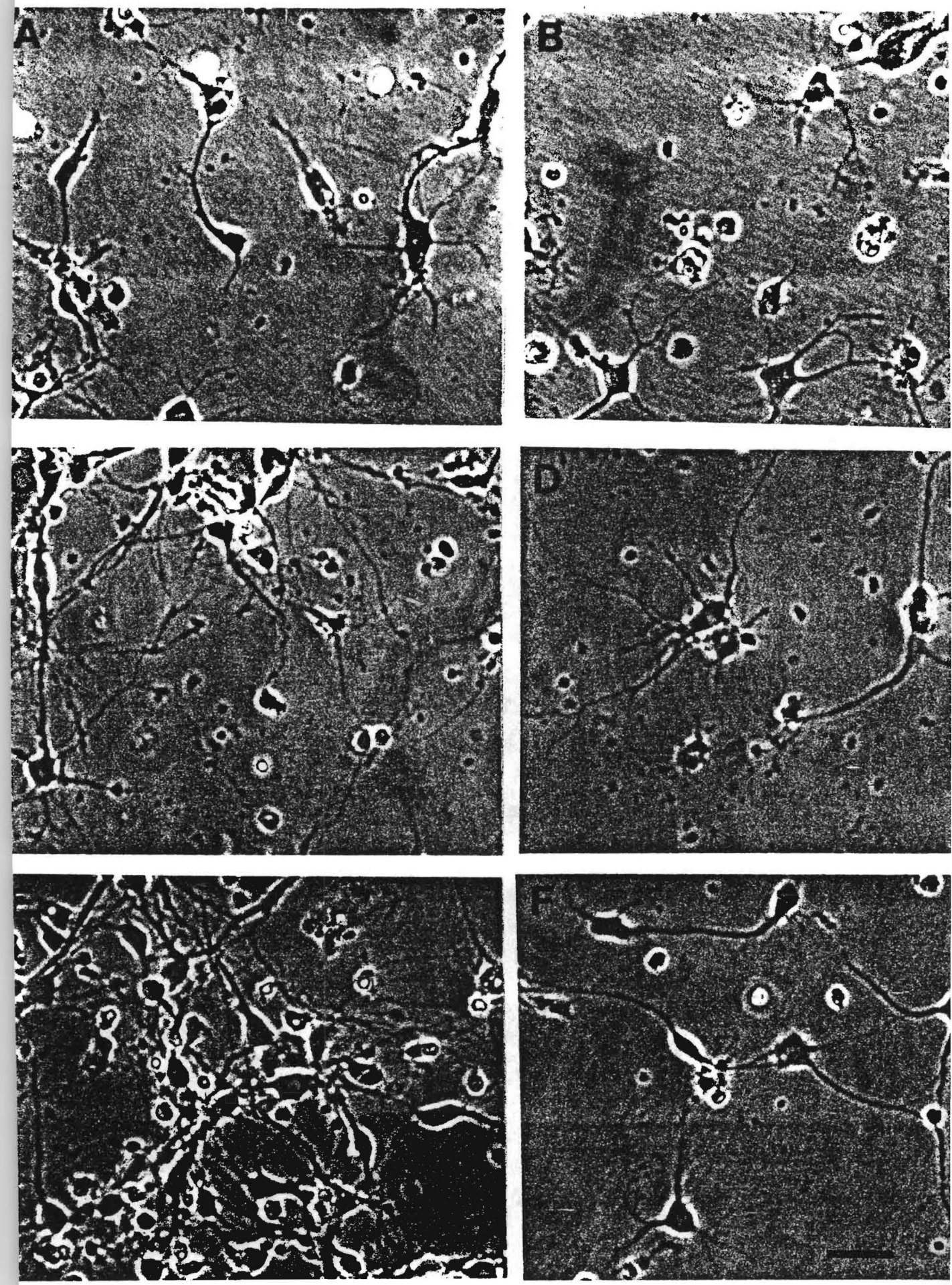
## Neuronal Responses to Irradiation

In preliminary experiments several dishes of freshly plated hippocampal neurons were placed on the larger brass antenna and were irradiated for four hours every 24 hours for three to four days. The carrier signal frequency was 400 MHz with an amplitude modulation of 16 Hz. Morphological examination revealed that cell viability was reduced in both irradiated and non-irradiated control plates. This effect was attributed to poor temperature regulation of the plates while they are removed from the incubator and placed on the antenna. Subsequently all cells were irradiated within the incubator itself.

Culture dishes continuously exposed to the RF field for four consecutive days develop density gradients, with a greater number of cells occurring in the center of the plate which is directly in the field as opposed to the more peripheral areas of the plate which are outside of the field. Non-irradiated control plates do not display this density gradient (Fig. 2). This finding has been replicated using exposure periods ranging from 15 min. to 14 days, with the plates being removed only briefly from the antenna for medium changing every 7 days. Durations longer than 14 days were not investigated because the number of neurons per dish declines normally with time in culture, and after two weeks about 25% of the cells originally plated are still present.

The density gradient is established within 20 min of placing the plates on the antennae. The concentration phenomenon is not

Figure 2. Effects of temperature radiofrequency radiation on neuronal morphology. Cells were either placed in a 37°C incubator, a 40°Cincubator, or on the RF antenna in a 37°C incubator from the time of plating through day 5. Photographs were then taken of cells using a phase contrast microscope at either center or periphery of the culture dish. A) 37°C center, B) 37° C periphery, C) 40° C center, D) 40° C periphery, E) RF center, and F) RF periphery.


A
B
D
F

neuronspecificsincesimilardensitygradientsareobservedwhen both HeLa cells and Chinese Hamster Ovary (CHO) cells are plated and exposed to the RF field for 20 min. Neurons exposed to the field for any period up to 14 days appear to be entirely normal morphologically using phase contrast microscopy. In fact, there appears to be even a greater density of neurites in the RF-exposed cells, and this may be a function of the higher temperature in the center of the dish.

## DISCUSSION

Cultured fetal hippocampal neurons were not adversely affected by continuous exposure to RF radiation in the 300 MHz to 470 MHz range. Although lacking a precise temperature reference for calibrating the vitek probe posed some difficulty, measurements consistently revealed that a 3° centigrade increase occurred in the culture dish directly on the axis of symmetry of the antennae. Temperature controls conducted in an incubator set at 40° C revealed that the fetal neurons survive and develop neurites quite well at this temperature, but that neurons and glia are both killed within 24 hours if they are kept at 42.4 C. These findings confirm that temperatures produced in the center of the RF field must be lower than 42° C. The most striking phenomenon was an obvious increase in the number of neurons within the RF field as opposed to the periphery of the culture dish. It was very exciting to think that perhaps the few neurons that were still dividing when plating occurred were somehow stimulated to continue division. However, this does not

appear to be the case. The fact that the concentration phenomenon occurred within 20 minutes of plating the cells indicates that the increase in cell number is not due to cell division since this period of time is too short for mitosis to occur. In experiments using continuously dividing cells such as HeLa and CHO cells, the same rate of division occurred in RF-exposed and control plates. In all cases the density gradient of cells did not occur if cells were allowed to fully attach to the dish prior to placement on the antenna. These results indicate that the increase in cell number at the center of RF_exposed dishes results from convection currents set up by a thermal gradient in which the temperature was higher in the center of the dish than at the periphery. Thus single cells floating in the medium might be drawn up by these currents and then have a greater probability of settling and attaching above the antenna. Although this is not a particularly exciting finding, it does provide a means of concentrating healthy neurons within a small area, which facilitates optical recording of neuronal networks. We are currently working on exploiting this technique.

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October 31, 1991

**DEPARTMENT OF THE AIR FORCE**
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Aeronautical Systems Division/PMRSC
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Attention: Mr. John M. Lipker

Reference: Task Order 0007 Under Contract No. F33615-87-D-0626, "Pre-Acquired Research in Biotechnology", Georgia Tech Project No. B-03-A07

Subject : Final Technical Report, Sequence No. 19, for the Reporting Period 1 September 1989 through 1 July 1990

Gentlemen:

A copy of the signed Form DD 250 has been received for the Final Technical Report (Draft No. 2) indicating that it has been accepted as submitted. This report automatically becomes the Final Technical Report for this Task Order; therefore, the requirement for a Final Technical Report on Task Order 0007 is met.

Sincerely,

J.C. Toler, Director
Project No. B-03-A07

cc: Dr. Deborah Armstrong, Univ. of TX
Dr. Jonathan Kiel, USAFSAM/RZP